Chapter 50 - **SANS FROM A PROTEIN COMPLEX**

1. INTRODUCTION

Proteins perform the basic tasks essential for life. Enzymes are proteins that catalyze reactions and perform specialized cell functions. Kinases are a large class of enzymes that add phosphate groups to proteins and other biomolecules. In bacteria, kinases coordinate cellular responses to external signals. The function of histidine kinases is to transfer a phosphate group from an ATP molecule to a specific amino acid (histidine) site on the kinase. This transfer mechanism is referred to as phosphorylation. The phosphate is further transferred from the histidine site to another protein.

A histidine kinase named KinA is essential in the sporulation mechanism of bacteria. Sporulation is the formation of spores which are capable of surviving dormant for a long time before reproducing again when external conditions become more favorable. KinA functions in concert with a response regulator named Sda which can halt sporulation when DNA damage is detected. Sda binds onto KinA to stop its autokinase activity thereby stopping its sporulation function by halting the phosphate transfer mechanism.

Histidine kinases are comprised of two domains: (1) a "sensor" domain which recognizes the sporulation signal and (2) an "autokinase" domain which performs the phosphotransfer function.

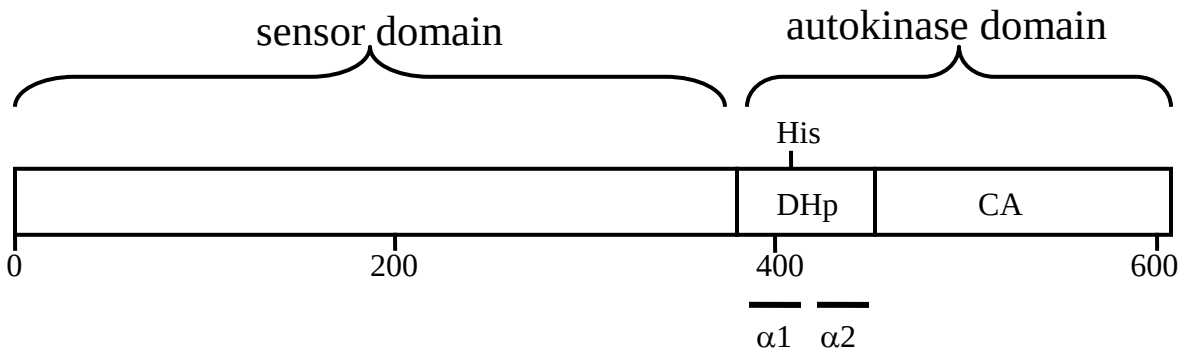

Figure 1: Representation of the two domains that form KinA. The 606 amino acid sequence is marked. The histidine used in the phosphate transfer mechanism is located in position 405.

The crystalline structures of KinA and of its response regulator Sda are known from x-ray crystallography. The structure of the KinA/Sda complex, however, is not known. The autokinase domain is divided into two sub-domains: two catalytic and ATP binding parts named the CA dimer and a histidine phosphotransfer part named the DHp stalk.

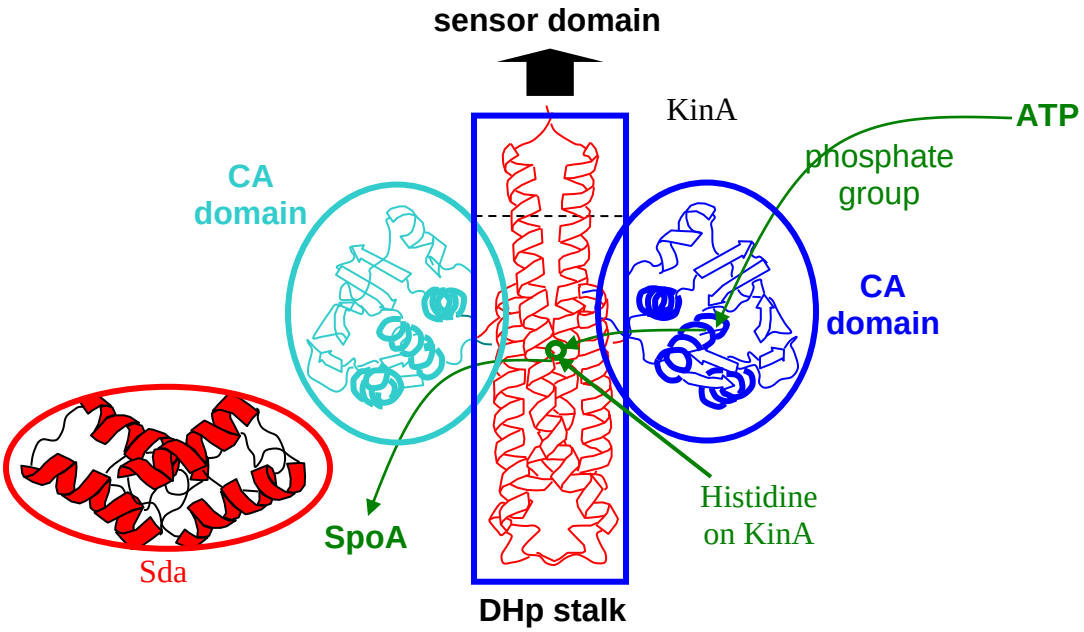

Figure 2: Representation of the dimeric KinA protein comprising the two CA domains and the DHp stalk. One of the two histidine amino acids used in the phosphate transfer mechanism is shown in green. The other one is behind. The response regulator Sda is also included on the left side.

KinA is comprised of 606 amino acid residues. The first 383 constitute the sensor domain with three PAS regions and the next 223 constitute the autokinase domain with residues 383-465 for the DHp region and residues 456-606 for the CA dimer region. The amino acid sequence for KinA has been only partly resolved.

Figure 3: Spelling out of the amino acid residue sequence (383-606) showing two helices α1 and α2 located in the DHp stalk. The histidine used in the phosphate transfer mechanism is shown at position 405. Dash marks correspond to the sequences that have not been resolved.

The DHp stalk is formed of a bundle of four helices. The two histidine phosphorylation active sites are located halfway along the stalk (helices α1 and α'1). When the sensor domain receives the signal to sporulate, each of the two CA domains transfers a phosphate group from an adenosine triphosphate (ATP) molecule to one of the two histidine active sites. This is performed through a hinge-like motion of the CA domains; these domains pivot to transfer the phosphate groups.

The response regulator Sda halts (inhibits) the sporulation mechanism performed by KinA when necessary. When Sda binds onto KinA, the phosphotransfer mechanism performed by the CA domains is stopped. The Sda binding region on KinA has been identified as located toward the lower part of the DHp stalk with no direct interaction with the CA domains.

The purpose of the investigations reported here is to understand the positioning of the KinA and Sda parts of the protein complex and to get insight into the sporulation inhibition mechanism (Whitten et al, 2007). This is performed using the SAXS and the SANS techniques.

2. SAXS FROM THE PROTEIN COMPLEX

The KinA and the Sda parts of the protein complex have been characterized by standard methods. The molecular weights have been determined using mass spectroscopy and size exclusion chromatography (SEC). These showed the dimer nature of Sda. Kinase assay identified the Sda surface involved in binding the KinA protein. Characterization was performed both on the protein complex and on its individual components.

SAXS was performed on KinA and Sda alone and on the KinA-Sda complex. Two protein complex concentrations in the dilute regime in water were measured.

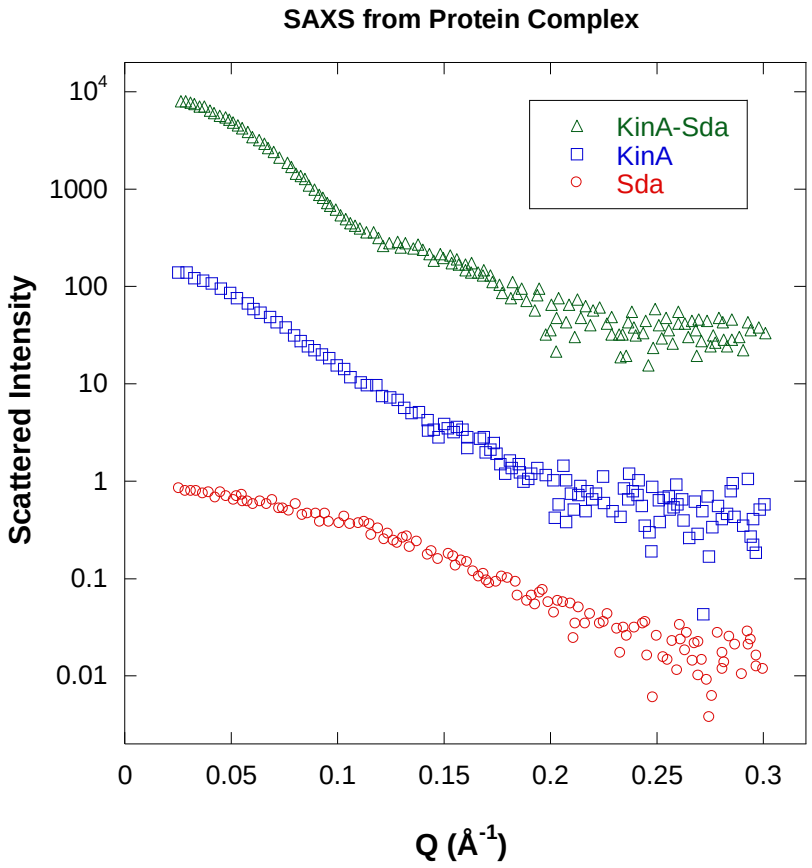

Figure 4: SAXS from dilute solutions of KinA and Sda alone and of the KinA-Sda complex. Curves have been shifted arbitrarily upward to avoid overlap.

The pair-distance probability distribution function $P(\vec{r})$ is the inverse Fourier transform of the scattering form factor P(Q).

$$P(Q) = \int d\vec{r} \exp(-i\vec{Q}.\vec{r})P(\vec{r}) = \int_0^\infty dr 4\pi r^2 \left(\frac{\sin(Qr)}{Qr}\right)P(\vec{r}). \qquad (1)$$

The distance distribution function $4\pi r^2 P(\vec{r})$ (also referred to as the pair correlation function) was obtained and plotted. It gives an estimate of the average size of the KinA, Sda and KinA/Sda complex (peak position) and goes to zero at the particle edge (at D_{max}).

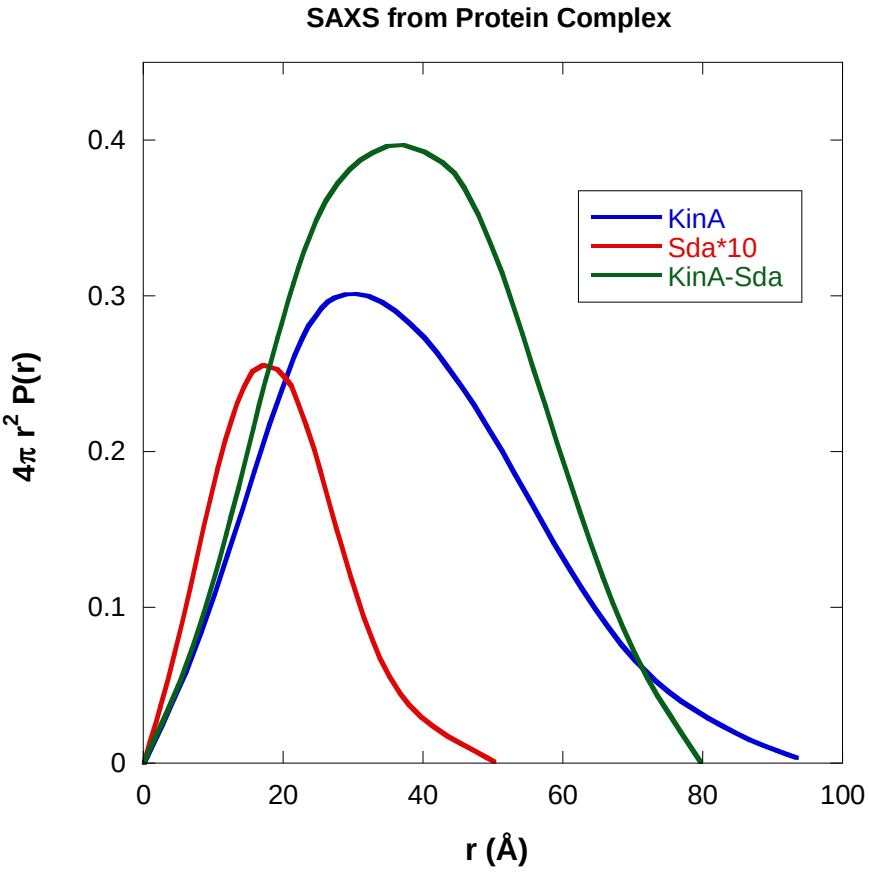

Figure 5: Distance distribution functions obtained from SAXS data from the individual protein and inhibitor and from the protein complex. Scattering from Sda was scaled up (*10).

The radius of gyration is obtained as the second moment of $P(\vec{r})$:

$$R_g^{\,2} = < r^2 > = \frac{\int_0^\infty dr 4\pi r^2 \, r^2 P(r)}{\int_0^\infty dr 4\pi r^2 \, P(r)}. \qquad (2)$$

R_gs for KinA and Sda were obtained from the Guinier analysis and from the second moment of P(r) analysis and are summarized in a table. Sizes obtained for the KinA/Sda complex showed a compaction of KinA after Sda binding.

Table 1: Size parameters derived from the SAXS Guinier and P(r) data analyses.

Sample	Concentration (mg/ml)	Guinier R_g (Å)	R_g from P(r) (Å)	D_{max} from P(r) (Å)
KinA	3.7	29.3	29.6	95

Sda	5.2	15.3	15.4	52
KinA/Sda	4.4	29.2	29.1	80
KinA/Sda	3.7	29.4	29.1	80

3. SANS FROM THE PROTEIN COMPLEX

SANS measurements were performed with deuterated Sda (d-Sda) complexed with non-deuterated KinA. Both KinA and Sda were overexpressed using E. coli cultures. D-Sda was obtained using a culture in d-water. Two dilute solution concentrations (3.5 mg/ml protein in 50 mM NaCl and 11.9 mg/ml protein in 200 mM NaCl) were measured. A contrast variation series was performed in each case using mixtures of water and d-water.

Radius of Gyration Analysis

Here also, radii of gyration were obtained for KinA, d-Sda and the KinA/d-Sda complex. In order to estimate the radius of gyration of the complex in terms of the individual radii of gyration, the following argument is used. Consider a compound object formed of two particles: particle 1 (for d-Sda) of volume V_1 and particle 2 (for KinA) of volume V_2 separated by a distance D (between their centers of mass) and define $\Delta\rho_1$ and $\Delta\rho_2$ for their excess scattering length densities ($\Delta\rho_1 = \rho_1-\rho_0$ and $\Delta\rho_2 = \rho_2-\rho_0$ where index 0 refers to the solvent).

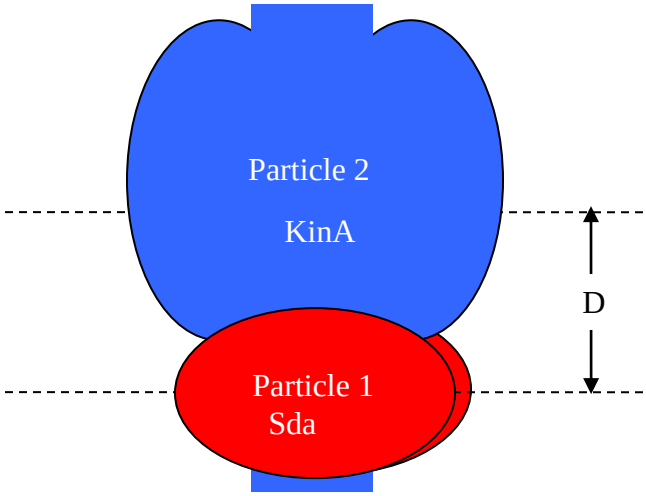

Figure 6: Schematic representation of a compound object formed of two distinct particles. The inter-distance between the centers of mass is D.

According to the parallel axis theorem, the radius of gyration of the compound object is expressed in terms of the individual radii of gyration R_{g1} and R_{g2} as follows:

$$R_g^{\,2} = \frac{\Delta\rho_1 V_1}{\Delta\rho_1 V_1 + \Delta\rho_2 V_2} R_{g1}^{\,2} + \frac{\Delta\rho_2 V_2}{\Delta\rho_1 V_1 + \Delta\rho_2 V_2} R_{g2}^{\,2} + \left(\frac{\Delta\rho_1 V_1}{\Delta\rho_1 V_1 + \Delta\rho_2 V_2}\right)\left(\frac{\Delta\rho_2 V_2}{\Delta\rho_1 V_1 + \Delta\rho_2 V_2}\right) D^2 \qquad (3)$$

After a few straightforward manipulations, this can be put into the following Stuhrmann relation (Ibel-Stuhrmann, 1975):

$$R_g^{\,2} = R_{gm}^{\,2} + \frac{\alpha}{\Delta\bar{\rho}} - \frac{\beta}{\Delta\bar{\rho}^2}. \qquad (4)$$

This is expressed in terms of the average neutron contrast $\Delta\bar{\rho}$ defined as:

$$\Delta\bar{\rho} = \frac{\Delta\rho_1 V_1 + \Delta\rho_2 V_2}{V_1 + V_2} \quad . \qquad (5)$$

The various parameters are defined as follows:

$$R_{gm}^{\,2} = \frac{V_1}{V_1 + V_2} R_{g1}^{\,2} + \frac{V_2}{V_1 + V_2} R_{g2}^{\,2} + \left(\frac{V_1}{V_1 + V_2}\right)\left(\frac{V_2}{V_1 + V_2}\right) D^2$$

$$\alpha = (\rho_1 - \rho_2)\left(\frac{V_1}{V_1 + V_2}\right)\left(\frac{V_2}{V_1 + V_2}\right)\left[R_{g1}^{\,2} - R_{g2}^{\,2} + \left(\frac{V_2^{\,2} - V_1^{\,2}}{(V_1 + V_2)^2}\right) D^2\right]$$

$$\beta = (\rho_1 - \rho_2)^2 \left(\frac{V_1}{V_1 + V_2}\right)^2 \left(\frac{V_2}{V_1 + V_2}\right)^2 D^2. \qquad (6)$$

Variation of the measured radius of gyration (squared) with $1/\Delta\bar{\rho}$ yields three parameters ($R_{gm}^{\,2}$, α and β or R_{g1}, R_{g2} and D). The various points were obtained from the contrast variation series. R_g^2 values were obtained from Guinier plots.

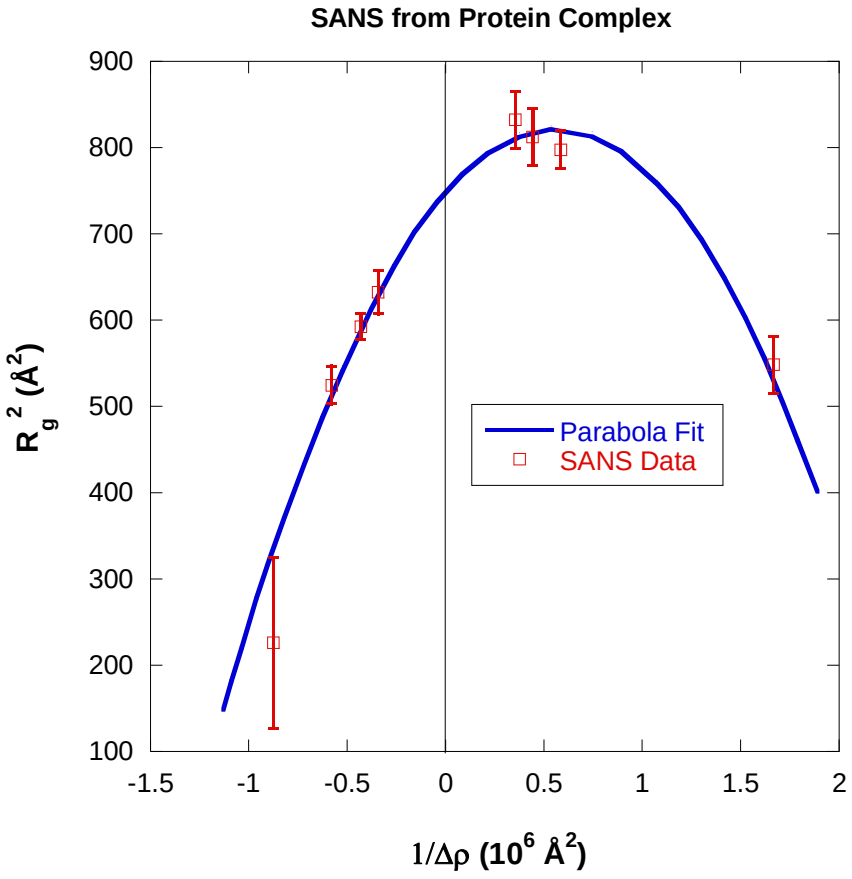

Figure 7: Stuhrmann plot for the KinA/d-Sda complex.

The positive sign for the coefficient α reveals that the higher contrast component (d-Sda) lies towards the periphery of the complex. The peak position (maximum) corresponds to the condition $\Delta\bar{\rho} = \alpha/2\beta$. Since $\beta > 0$, the condition $\alpha > 0$ implies:

$$\left(\frac{V_2^{\,2} - V_1^{\,2}}{(V_1 + V_2)^2}\right) D^2 > \left(R_{g2}^{\,2} - R_{g1}^{\,2}\right). \qquad (7)$$

Moreover, it was found that the distance between the centers of mass is D = 27.0 Å along with R_{g1} = 25.3 Å for d-Sda and R_{g2} = 25.4 Å for KinA.

Pair Correlation Function Analysis

The SANS intensity for the contrast variation series from the KinA/d-Sda complex can be modeled as:

$$I(Q) = \Delta\rho_1^{\,2} I_{11}(Q) + \Delta\rho_2^{\,2} I_{22}(Q) + \Delta\rho_1 \Delta\rho_2 \, 2I_{12}(Q). \qquad (8)$$

$I_{11}(Q)$ is the scattering from particle 1, $I_{22}(Q)$ is the scattering from particle 2 and $I_{12}(Q)$ is the cross term representing correlations between a pair of scatterers belonging to the two particles.

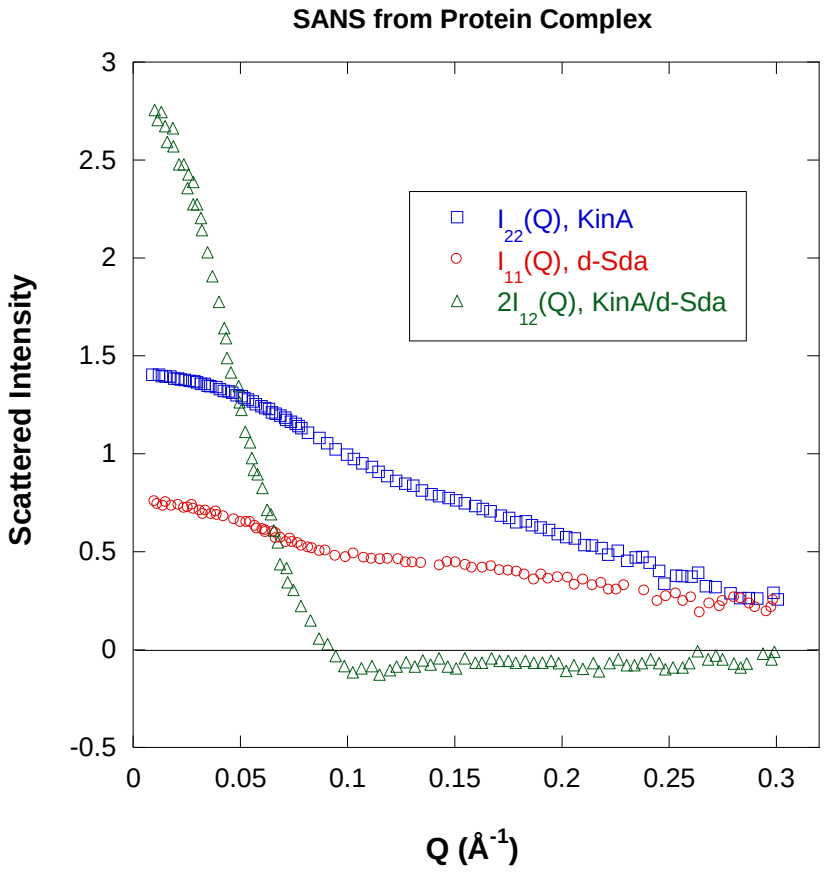

Figure 8: Single-particle and inter-particle form factors $I_{11}(Q)$, $I_{22}(Q)$ and $I_{12}(Q)$ obtained from SANS measurement. Scattered intensity is in arbitrary units.

The inverse Fourier transform for the self and cross terms has been obtained and plotted.

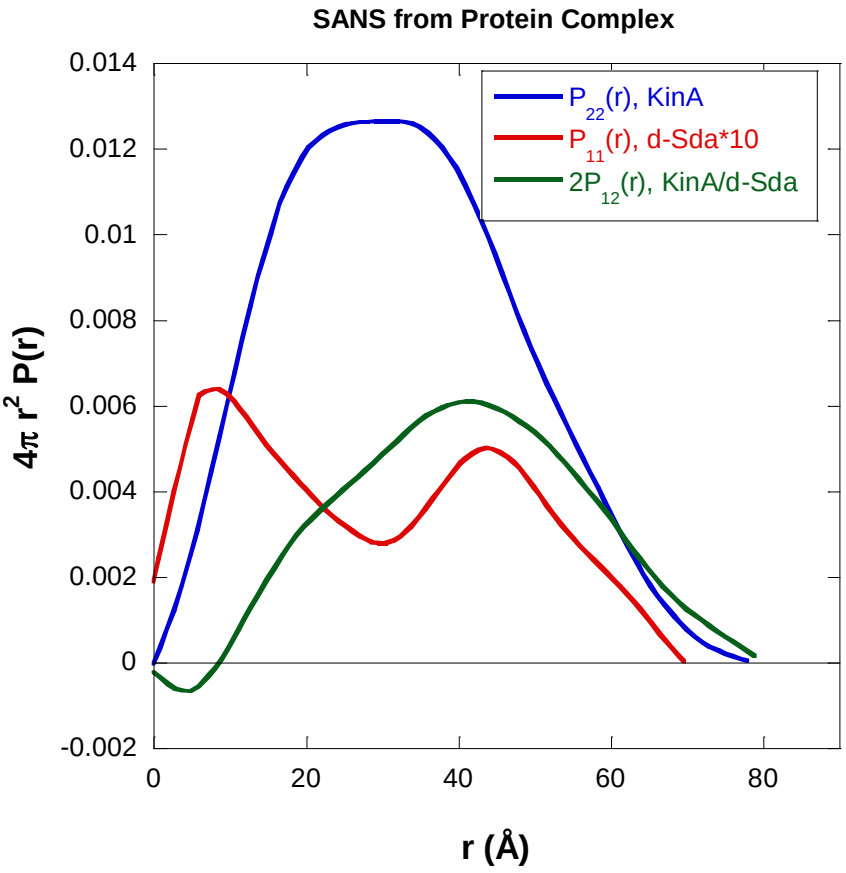

Figure 9: Single-particle and inter-particle pair correlation functions $P_{11}(r)$, $P_{22}(r)$ and $P_{12}(r)$ obtained as the inverse Fourier transform of the form factors.

The pair distribution function for the d-Sda component is characterized by two peaks. Although d-Sda forms dimers when it is alone in solution, it binds to KinA as two individual distinct monomers. The two peaks indicate that centers of mass of the two d-Sda monomers are separated by 45 Å. The KinA and the d-Sda molecules themselves are separated by 27 Å. It is noted that the KinA molecule appears more compact when d-Sda is bound to it. Size parameters obtained from SANS data analyses are summarized in a table.

Table 2: Size parameters derived from the SANS Guinier and P(r) analyses.

Sample	Concentration (mg/ml)	% H_2O	Guinier R_g (Å)	P(r) R_g (Å)	P(r) D_{max} (Å)
KinA/d-Sda	3.7	0	28.9	28.3	80
		10	28.3	28.5	80
		20	28.0	27.9	80
		80	22.5	22.7	80
		90	27.1	24.4	80
		100	25.7	25.1	80

KinA/d-Sda	11.9	0	28.3	28.9	80
		10	28.2	28.3	80
		20	29.1	28.2	80
		80	22.5	23.0	70
		90	24.3	24.2	75
		100	25.3	25.1	75
KinA/d-Sda	26.6	40	26.1	23.4	70

The cross term $P_{12}(r)$ (inverse Fourier transform of $I_{12}(Q)$) is characterized by one peak. This means that the two d-Sda monomers lie approximately equidistant from the two CA catalytic domains and must be located on opposite sides of the lower part of the DHp stalk. Even though the d-Sda monomers are nowhere near the CA domains, they affect their phosphorylation function. This must be performed through a remote (called allosteric) control via the four helix bundle. This bundle is probably disturbing the hinge motion of the CA domains necessary for the transfer of a phosphate group from an ATP molecule to the histidine site in the middle of the DHp stalk. The d-Sda inhibitor is therefore not acting as a molecular barrier to the autokinase motion of the CA domains.

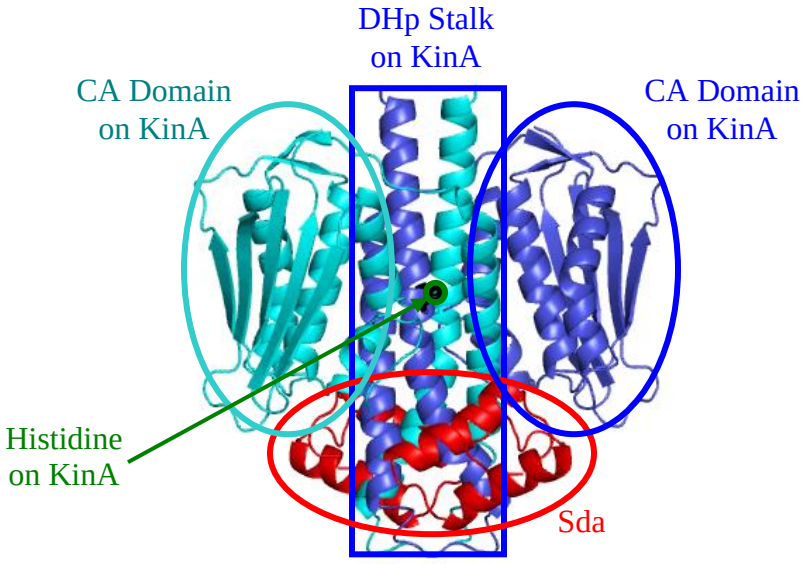

Figure 10: Computer rendering of the KinA/Sda protein complex consistent with the scattering results obtained. The second histidine and the second Sda monomer located on the back are not shown.

4. COMMENTS

The structure of amorphous protein complexes are hard to resolve since no single-crystal can be obtained and therefore no diffraction data can be obtained. Small-angle scattering helps resolve the main structural characteristics of such protein complexes. The SANS technique when used with contrast variation and deuterated macromolecules can map out sizes and inter-particle distances between the various components forming the complex. Information obtained from scattering methods and from other techniques helps in the understanding of the mechanisms involved.

A histidine kinase KinA and its inhibitor Sda have been investigated using SAXS and SANS. This protein complex is relevant to the sporulation mechanism in bacteria. Sporulation happens through the transfer of a phosphate group from an ATP molecule to a specific histidine site on the KinA stalk. This is performed by the hinge-like motion of the two CA domains of KinA. The phosphate is further transferred from the histidine site to a sporulation protein (SpoA) that docks onto KinA. The inhibition of the sporulation mechanism is performed when protein Sda forms a complex with KinA. Even though Sda is located at the bottom of the DHp stalk, it stops the sporulation mechanism of the KinA CA domains allosterically (i.e., remotely) through conformational changes in the four helix bundle of the DHp stalk.

REFERENCES

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K. Ibel, H.B. Stuhrmann, "Comparison of Neutron and X-ray Scattering of Dilute Myoglobin Solutions", Journal of Molecular Biology 93, 255-265 (1975)

QUESTIONS

1. What is a histidine?
2. What is a kinase?
3. What is an inhibitor? Name a kinase protein and its inhibitor.
4. What is sporulation?
5. What is the difference between a spore and a seed?
6. What is measured through the Guinier plot?
7. What is the pair correlation function $P(\vec{r})$? How about the size distribution function?
8. State the so-called Stuhrmann relation used to analyze SANS data.
9. What is the parallel axis theorem for the radius of gyration? What else is it used for?
10. How are deuterated proteins obtained?

ANSWERS

1. Histidine is one of the 20 amino acid residues. These are the building blocks for proteins.
2. A kinase is a protein that performs the function of phosphorylation. Phosphorylation is the transfer of a phosphate group from an ATP molecule to an active site on the kinase or other molecules.
3. An inhibitor is a protein that stops (inhibits) a specific function. KinA is a histidine kinase used in the sporulation function and Sda is its inhibitor; i.e., it stops that function.
4. Sporulation consists in the formation of spores which package the genetic material of a species. Spores are capable of surviving for a long time before reproducing when external conditions become more favorable.
5. Spores contain the genetic material for reproduction only. Seeds contain the genetic material as well as food for initial growth.
6. The Guinier plot measures the radius of gyration which is a measure of the particle density distribution around the center-of-mass.
7. The pair correlation function $P(\vec{r})$ is the inverse Fourier transform of the scattering intensity (form factor). It is the probability of finding a scatterer at position $\vec{r}$ given that there is a scatterer at the origin. The size distribution function is defined as $4\pi r^2 P(\vec{r})$.
8. The Stuhrmann relation is used to analyze R_g data when a contrast variation series is measured. It related R_g^2 to the average excess scattering length density $\Delta\bar{\rho}$ as follows:

$R_g^2 = R_{gm}^2 + \frac{\alpha}{\Delta\bar{\rho}} - \frac{\beta}{\Delta\bar{\rho}^2}$. R_{gm}^2, α and β are related to structural parameters.

9. The parallel axis theorem relates the radius of gyration of a compound particle to the radii of gyration of the individual components. The parallel axis theorem is used in cases where the second moment is used. The moment of inertia for compound particles is also calculated that way.
10. Deuterated proteins are obtained from E. coli cultures grown (overexpressed) in d-water.